

Appl. No. 10/080,428
Resp. to Not. To Comply filed May 19, 2005
Resp. to Non Compliant Amdt. dated April 19, 2005

Listing of Claims

1. (Currently Amended) An eukaryotic host cell genetically engineered to ~~activate the~~ express a p65 NF-kappa-B transcription factor complex, and to express a protein of interest as an extracellular product.
- 2-3. (Canceled)
4. (Currently Amended) The host cell of claim 2-1 wherein the p65 NF-kappa-B transcription factor is expressed under control of a heterologous regulatory element.
5. (Original) The host cell of claim 4, wherein the heterologous regulatory element is a viral promoter.
6. (Original) The host cell of claim 5, wherein the viral promoter is selected from the group consisting of a CMV promoter, an SV40 promoter, an RSV promoter and an adenoviral promoter.
7. (Original) The host cell of claim 1, wherein the protein of interest is selected from the group consisting of a soluble TNF receptor, a soluble IL-4 receptor, a soluble IL-1 type II receptor, a soluble Flt3 ligand, a soluble CD40 ligand, CD39, CD30, CD27, a TEK/Ork, IL-15, a soluble IL-15 receptor, Ox 40, GM-CSF, RANKL, RANK, TRAIL, a soluble TRAIL receptor, tissue plasminogen activator, Factor VIII, Factor IX, apolipoprotein B, apolipoprotein A-I, an IL-2 receptor, an IL-2 antagonist, alpha-1 antitrypsin, calcitonin, growth hormone, insulin, insulinotropin, insulin-like growth factors, parathyroid hormone, interferons, superoxide dismutase, glucagon, an erythropoietin, an antibody, glucocerebrosidase, an Fc-fusion protein, globins, nerve growth factors, interleukins, colony stimulating factors, and a cytokine.
8. (Original) The host cell of claim 1, wherein the host cell is further genetically engineered to express a selectable marker.
9. (Original) The host cell of claim 1, wherein the host cell is a mammalian cell.
10. (Original) The host cell of claim 9, wherein the host cell is selected from the group consisting of CHO, VERO, BHK, HeLa, CV1, COS, MDCK, 293, 3T3, myeloma, PC12 and WI38.
11. (Original) The host cell of claim 1, wherein the host cell is adapted to grow in protein-free medium.
12. (Currently Amended) The host cell of claim 21, wherein the p65 NF-kappa-B transcription factor is a caspase resistant p65 mutant.
13. (Currently Amended) The host cell of claim 21, wherein the host cell is genetically engineered to express a second NF-kappa-B transcription factor.

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14. (Withdrawn) A method of producing a protein of interest, the method comprising culturing an eukaryotic host cell genetically engineered to activate the NF-kappa-B transcription factor complex, and to express a protein of interest as an extracellular product, under conditions such that the protein of interest is expressed and secreted.
15. (Withdrawn) The method of claim 14, further comprising collecting the protein of interest.
16. (Withdrawn) The method of claim 14, wherein the host cell is genetically engineered to express an NF-kappa-B transcription factor.
17. (Withdrawn) The method of claim 16, wherein the NF-kappa-B transcription factor is selected from the group consisting of p65, p50, cRel, p52 and RelB.
18. (Withdrawn) The method of claim 16, wherein the NF-kappa-B transcription factor is expressed under control of a heterologous regulatory element.
19. (Withdrawn) The method of claim 18, wherein the heterologous regulatory element is a viral promoter.
20. (Withdrawn) The method of claim 19, wherein the viral promoter is selected from the group consisting of a CMV promoter, an SV40 promoter, an RSV promoter and an adenoviral promoter.
21. (Withdrawn) The method of claim 14, wherein the protein of interest is selected from the group consisting of a soluble TNF receptor, a soluble IL-4 receptor, a soluble IL-1 type II receptor, a soluble Flt3 ligand, a soluble CD40 ligand, CD39, CD30, CD27, a TEK/Ork, IL-15, a soluble IL-15 receptor, Ox 40, GM-CSF, RANKL, RANK, TRAIL, a soluble TRAIL receptor, tissue plasminogen activator, Factor VIII, Factor IX, apolipoprotein E, apolipoprotein A-I, an IL-2 receptor, an IL-2 antagonist, alpha-1 antitrypsin, calcitonin, growth hormone, insulin, insulinotropin, insulin-like growth factors, parathyroid hormone, interferons, superoxide dismutase, glucagon, an erythropoietin, an antibody, glucocerebrosidase, an Fc-fusion protein, globins, nerve growth factors, interleukins, colony stimulating factors, and a cytokine.
22. (Withdrawn) The method of claim 14, wherein the host cell is further genetically engineered to express a selectable marker.
23. (Withdrawn) The method of claim 14, wherein the host cell is a mammalian cell.
24. (Withdrawn) The method of claim 23, wherein the host cell is selected from the group consisting of CHO, VERO, BHK, HeLa, CV1, MDCK, 293, 3T3, myeloma, PC12 and WB38.
25. (Withdrawn) The method of claim 14, wherein the host cell is cultured in protein-free medium.

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26. (Withdrawn) The method of claim 15, wherein the NF-kappa-B transcription factor is a caspase resistant p65 mutant.
27. (Withdrawn) The method of claim 14, wherein the host cell is transiently transfected.
28. (Withdrawn) The method of claim 14, wherein the host cell is stably transformed.
29. (Withdrawn) The method of claim 15, wherein the host cell is genetically engineered to express a second NF-kappa-B transcription factor.
30. (Withdrawn) A method of producing an eukaryotic cell for production of a protein of interest, the method comprising genetically engineering an eukaryotic cell to express a gene that encodes a protein of interest as an extracellular product, and to activate the NF-kappa-B transcription factor complex.
31. (Withdrawn) A method of producing a mammalian cell line capable of growth in protein-free medium, the method comprising exposing cells that have been genetically engineered to activate the NF-kappa-B transcription factor complex to protein-free medium, and isolating a cell line that grows in protein-free medium.
32. (Withdrawn) The method of claim 31, further comprising exposing the cells to peptone-free medium, and isolating a cell line that grows in peptone-free medium.
33. (Withdrawn) A method of producing an eukaryotic cell for production of a protein of interest, the method comprising genetically engineering an eukaryotic cell to express a protein of interest as an extracellular product, wherein the eukaryotic cell has been genetically engineered to activate the NF-kappa-B transcription factor complex.
34. (Withdrawn) A method of producing an eukaryotic cell for production of a protein of interest, the method comprising genetically engineering a cell to activate the NF-kappa-B transcription factor complex, wherein the eukaryotic cell expresses a protein of interest as an extracellular product.